

## N-partitioning, nitrate reductase and glutamine synthetase activities in two contrasting varieties of maize<sup>(1)</sup>

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**Abstract** – In order to identify useful parameters for maize genetic breeding programs aiming at a more efficient use of N, two maize varieties of contrasting N efficiency, Sol da Manhã NF (efficient) and Catetão (inefficient) were compared. Experiments were carried out under field and greenhouse conditions, at low and high N levels. The parameters analysed included total and relative plant and grain N content, biomass and the activities of nitrate reductase and glutamine synthetase in different parts of the plant. It was found that the translocation efficiency of N and photoassimilates to the developing seeds and the source-sink relations were significantly different for the two varieties. N content of the whole plant and grain, cob weight and the relative ear dry weight were useful parameters for characterizing the variety Sol da Manhã NF as to its efficient use of N. Enzymes activity of glutamine synthetase (transferase reaction) and nitrate reductase did not differ among the varieties.

**Index terms:** nutrient transport, nitrogen, grain, source sink relations, plant breeding.

### Partição de nitrogênio e atividade das enzimas nitrato redutase e glutamina sintetase em duas cultivares contrastantes de milho

**Resumo** – Com o objetivo de identificar parâmetros que possam ser utilizados em programas de melhoramento genético em milho para uso eficiente de N, duas cultivares de milho contrastantes quanto ao uso deste nutriente, Sol da Manhã NF (eficiente) e Catetão (não-eficiente), foram avaliadas em dois experimentos conduzidos no campo e em casa de vegetação, respectivamente, sob nível baixo e alto de N. Os caracteres avaliados foram: teor e conteúdo de N em diferentes partes da planta; massa seca; peso dos grãos e de diferentes partes da planta; biomassa, e atividade das enzimas nitrato redutase e glutamina sintetase. O mecanismo de translocação de N e de fotoassimilados para os grãos e a relação fonte/dreno foram importantes para diferenciar a cultivar eficiente da não-eficiente. Conteúdo de N nos grãos e total das plantas, peso do sabugo e relação peso de espiga/matéria seca foram importantes para caracterizar a cultivar Sol da Manhã NF eficiente no uso do N. A atividade das enzimas glutamina sintetase (reação da transferase) e nitrato redutase não foram eficientes para discriminar as cultivares em estudo.

**Termos para indexação:** transporte de nutrientes, nitrogênio, grãos, relação fonte-dreno, melhoramento de plantas.

## Introduction

Nitrogen (N) is the most expensive nutrient and the one required in largest quantity by the majority of crops, especially maize. Although present at high levels in soils, the amounts of N in mineral forms are generally low. It is commonly observed that in natural ecosystems a continued loss of N occurs not only through its removal by plants but also by leaching, volatilization and denitrification. It has been estimated

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that only 40% to 50% of the N applied as fertilizer is absorbed by the maize crop under tropical conditions (Peoples et al., 1995). The availability of N to plants in tropical climates is also impaired by environmental stress such as drought, waterlogging, low fertility soils, aluminium, among others (Magalhães & Fernandes, 1993; Machado & Magalhães, 1995).

Variations in N availability can affect plant development and grain production in maize (McCullough et al., 1994; Uhart & Andrade, 1995b). The effect of N availability on grain yields of maize can be assessed by physiological components such as the interception and efficient use of radiation and the partitioning of N to reproductive organs (Uhart & Andrade, 1995b). Parameters such as leaf area index, longevity of the leaf canopy and the efficient use of light in maize are all increased by N (Muchow & Davis, 1988). It is also known that both deficiency and excess N affect the partitioning of assimilates between vegetative and reproductive organs (Donald & Hamblin, 1976). N deficiency affects the supply of assimilates to the ear mainly through reductions in leaf area, photosynthetic activity and light absorption (Lemcoff & Loomis, 1986; Uhart & Andrade, 1995b).

The flow and remobilization of carbon (C) and N to the kernels during the grain filling period depends on the source-sink relationship, which is influenced by genotype-environment interactions, that among others may be altered by management factors such as planting date, population density, nutrients and water (Uhart & Andrade, 1995a). Furthermore, stress conditions can undoubtedly act directly on availability, assimilation and metabolism of N, interfering with the activity of enzymes of N metabolism and probably the catabolism of amino acids, proteins and other nitrogenous compounds (Machado et al., 1992; Magalhães & Fernandes, 1993). Genetic improvement trials, where tolerance to abiotic and biotic stresses are focused, can result in the selection of genotypes that are more efficient to scavenge and use N under stress conditions (Tollenaar et al., 1991; McCullough et al., 1994).

The selection of genotypes with a more efficient mechanism of N uptake and metabolism is a strategy aimed at increasing N utilization efficiency of the maize crop. Several trials for efficient use of N un-

der conditions of low N availability have been carried out with maize (Thiraporn et al., 1987; Eghball & Maranville, 1991; Machado et al., 1992). In order to characterize and select genotypes for efficient use of N, several authors have used physiological and biochemical parameters, such as high nitrate in leaves (Molarette et al., 1987), increased nitrate reductase activity (Cregan & Berkum, 1984; Feil et al., 1993), glutamine synthetase activities (Machado et al., 1992; Magalhães et al., 1993; Machado & Magalhães, 1995), or increased mobilization of N from leaves and stems to the kernels (Eghball & Maranville, 1991).

The objective of this study was to identify morphological, physiological and biochemical parameters that could be used to help genetic improvement programs to get maize genotypes more efficient in the use of N.

## Material and Methods

### Plant material

The two maize varieties used in this study, Sol da Manhã NF and Catetão, are considered to be highly and poorly efficient in the use of N, respectively (Machado, 1997). Catetão is a local variety with flint and dark orange kernel and whose germplasm is derived mainly from Cateto race (Paterniani & Goodman, 1978; Soares et al., 1998). Sol da Manhã NF has semi to flint kernels, yellow-orange showing segregation for white kernels. Its germplasm is derived from 36 populations of Central and South America mainly from Cateto, Eto, and Flints from the Caribbean (Machado, 1997). Both varieties contain germplasm of similar origin. Although the variety Catetão is little used today in view of its low yields, in this study it was useful as a control to evaluate the evolution of efficiency from an old to a more modern variety (Sol da Manhã NF).

### Experimental design and analytical methods

Two trials were carried out, one in field and another in greenhouse. The experimental design was a factorial with treatments arranged in a randomized complete block design with four replications. The data were subjected to analysis of variance and when F was significant at ( $p < 0.05$ ) the LSD (least significant difference) was applied.

The field experiment was carried out in tanks measuring 20 x 3 m and 2 m deep, containing a Red-Yellow Pod-

zolic soil, limed and fertilized as indicated by soil analysis. Each plot consisted of a 3 m row. After thinning, the final stand was 15 plants per plot. N fertilizer for the low N level treatment was all applied at planting, at the rate of 10 kg/ha of N in the form of urea. For the high N level treatment, 100 kg/ha of N was applied: 1/3 at planting, and 2/3 at 45 days after planting, as urea.

At harvest, kernel moisture and grain weight were determined. At 55 days after planting, the activities of the enzymes nitrate reductase and glutamine synthetase (transferase assay) were determined. Samples of tissues were removed from the first leaf above and opposite to the superior ear, according to Reed et al. (1980). Sample of 0.5 g of tissue were used. The method is described below.

After harvest, plants were taken to the laboratory and separated into husk, ear, grain and tassel. The leaves and stems were separated into three parts, denominated lower, mid, and upper. The leaves of the mid region were those present at two nodes above and two below the upper ear, and the corresponding stem represented the mid stem. The leaves of the lower region were those situated immediately below the mid-region, and the upper region those situated immediately above the mid-region. Following separation, the plant parts were dried in an oven at 70°C for three days and finally weighed and grounded. N was determined in a sample of 200 mg by Kjeldahl digestion followed by distillation and titration, according to Bremner & Mulvaney (1982).

N content was determined in different parts of the plant, defined as:  $N_{LL}$  = lower leaf nitrogen;  $N_{Lst}$  = lower stem nitrogen;  $N_{LSh}$  = lower shoot nitrogen ( $N_{LL} + N_{Lst}$ );  $N_{ML}$  = mid leaf nitrogen;  $N_{Mst}$  = mid stem nitrogen;  $N_H$  = husk nitrogen;  $N_{MSh}$  = mid shoot nitrogen ( $N_{ML} + N_H + N_{Mst}$ );  $N_{UL}$  = upper leaf nitrogen;  $N_{Ust}$  = upper stem nitrogen;  $N_{Tas}$  = tassel nitrogen;  $N_{USh}$  = upper shoot nitrogen;  $N_{Cob}$  = cob nitrogen;  $N_G$  = grain nitrogen;  $N_{Pl}$  = plant nitrogen (shoot minus grains) and  $N_T$  = total plant nitrogen;  $N_s$  = fertilizer applied.

Based on the model proposed by Moll et al. (1982), N use efficiency was defined as grain production per unit of N available in the soil. N use efficiency is  $Wg/Ns$  in which  $Wg$  is grain weight and  $Ns$  is N supply expressed in the same unit (e.g., g/plant). There are two primary components of N use efficiency: 1) the efficiency of absorption (uptake), and 2) the efficiency with which the N absorbed is utilized to produce grain. These are expressed as follows: uptake efficiency =  $Nt/Ns$  and utilization efficiency =  $Wg/Nt$ , where  $Nt$  is total N in the plant at maturity. Utilization efficiency,  $Wg/Nt$ , can be expressed as:  $Wg/Nt \equiv (Wg/Ng)$  and  $Ng/Nt \equiv (Na/Nt)(Ng/Na)$  in which:  $Wg/Ng$  = grain produced per unit of grain N;

$Ng/Nt$  = fraction of total N that is translocated to grain;  $Na/Nt$  = fraction of total N that is accumulated after silking;  $Ng/Na$  = ratio of N translocated to grain to N accumulated after silking.

For the greenhouse experiment the same statistical design was followed, using four replications. The experimental plot was made up of pots with four plants. The pots (3 L) contained vermiculite with 10 plants, initially. After 14 days plants were removed to leave four plants/pot. Pots were irrigated with nutrient solution from the seventh day after planting, using stocks I (Hoagland normal solution) and II (Hoagland solution without N). The treatments with high N levels received 100 mg of N/week and the low N treatments, 10 mg of N/week.

Twenty-five days after planting, the activities of the enzymes nitrate reductase and glutamine synthetase (transferase reaction) were determined in the leaves, using the third expanded leaf (top down) of all four plants in the pot. Glutamine synthetase was also determined in the roots of the same four plants. After washing and drying with a paper towel, the roots were cut to obtain the middle portion of the root system.

#### Enzyme analyses

In both experiments (field and greenhouse), the *in vivo* nitrate reductase assay was carried out according to Reed et al. (1980), whereby the plant tissue (0.5 g) was vacuum infiltrated with a solution containing nitrate. The nitrite produced was determined after diffusion to the assay medium. The incubation medium contained 0.1 M phosphate buffer pH 7.5, 1% n-propanol and 0.1 M  $KNO_3$ . After 10 and 40 min, 0.2 mL aliquots were removed for nitrite determination, by mixing with 1.8 mL of  $H_2O$ , 1.0 mL of 1% sulfanilamide in 1.5 N HCl and 1.0 mL of 0.02% N-(1-naphthyl)-ethylenediamine dihydrochloride. The change in absorbance at 540 nm was used to calculate the amount of nitrite produced.

Glutamine synthetase activity was carried out also in both experiments; it was determined in leaf (field and greenhouse experiments) and root tissue (greenhouse experiment) with samples of 0.5 g. These samples were ground in a chilled mortar with 10 volumes of 0.1 M imidazole-HCl buffer, pH 7.8, containing 1 mM DTT. The extract was filtered through muslin cloth and centrifuged at 15,000 g for 15 minutes at 2°C. An aliquot of the supernatant was assayed by the transferase reaction according to Rhodes et al. (1975). The assay mixture contained 65 mM glutamine, 17 mM hydroxylamine, 33 mM Na arsenate, 4 mM  $MnCl_2$ , 1.7 mM ADP and 100 mM imidazol buffer, pH 6.8, together with 0.2 mL of the extract in a final volume of 1.0 mL. The reaction was incu-

bated at 32°C for 30 min. Then 1 mL of FeCl<sub>3</sub> reagent (0.67 N HCl, 0.2 N TCA and 0.37 M FeCl<sub>3</sub>) were added, centrifuged and the colour read at 535 nm against a blank (Ferguson & Sims, 1971). Data are expressed as  $\mu\text{mol}$  glutamyl hydroxamate formed per hour per gram of fresh tissue.

## Results and Discussion

The level of N in the different parts of the plant are shown in Tables 1 and 2. Catetão had greater accumulation of N in the leaves and stems of the lower and mid regions, whereas Sol da Manhã NF presented greater accumulation in the cob and grain as well as the highest total N accumulation, indicating higher N uptake from the soil. These data indicate great dif-

ferences regarding the efficiency of two processes: the first involving the differential source-sink rate of the genotypes and the second the translocation of N. In this study, the variety Sol da Manhã had greater sink capacity with higher accumulation of N in the grain and better translocation capacity as compared to Catetão, which on the other hand accumulated more N in the leaves and stem (source).

Pan et al. (1995) suggest that absorption of N is regulated by the sink capacity of the ear and by the supply of photoassimilates. Moll et al. (1987) relate the efficiency of absorption and utilization of N and carbon in maize genotypes to a greater or lesser sink capacity. The data presented in this paper are consistent with the literature cited above, where the source/sink ratio was important in the characteriza-

**Table 1.** Mean N content of lower leaves ( $N_{LL}$ ), lower stems ( $N_{LS}$ ), lower shoot ( $N_{LSH}$ ) (leaves + stems), mid leaves ( $N_{ML}$ ), mid stem ( $N_{MS}$ ), husks ( $N_H$ ), mid shoot ( $N_{MSH}$ ) (leaves + stems + husks), upper leaves ( $N_{UL}$ ), upper stem ( $N_{US}$ ), tassel ( $N_{Tas}$ ) and upper shoot ( $N_{USH}$ ) (leaves + stems + tassel) in kg/ha, for the varieties Sol da Manhã NF and Catetão at two levels of N, one low (10 kg/ha) and the other high (100 kg/ha). Seropédica, RJ, Brazil, 1994.

Variety / N level	$N_{LL}$	$N_{LS}$	$N_{LSH}$	$N_{ML}$	$N_{MS}$	$N_H$	$N_{MSH}$	$N_{UL}$	$N_{US}$	$N_{Tas}$	$N_{USH}$
Sol da Manhã NF/ 10 kg/ha	0.270	2.178	2.448	1.645	2.420	0.718	4.782	1.290	1.090	0.733	3.113
Sol da Manhã NF/ 100 kg/ha	0.655	3.473	4.128	2.450	3.147	0.867	6.465	1.705	1.282	0.960	3.947
Catetão / 10 kg/ha	0.657	4.498	5.155	2.038	3.080	1.332	6.450	1.183	1.147	0.635	2.965
Catetão / 100 kg/ha	1.028	5.977	7.005	3.270	5.748	1.558	10.575	2.108	1.735	1.190	5.033
Mean	0.653	4.031	4.684	2.351	3.599	1.119	7.068	1.571	1.314	0.879	3.764
LSD (5%)	0.327	1.896	2.164	0.647	1.639	0.485	2.393	0.864	0.743	0.378	1.813
Mean for Sol da Manhã NF	0.463	2.825	3.275	2.047	2.784	0.792	5.624	1.498	1.186	0.846	3.530
Mean for Catetão	0.843**	5.238**	6.080**	2.654*	4.414*	1.445**	8.512**	1.645	1.441	0.912	3.999
Mean for 10 kg/ha of N	0.464	3.338	3.801	1.841	2.750	1.025	5.616	1.236	1.119	0.684	3.039
Mean for 100 kg/ha of N	0.841**	4.725*	5.554*	2.860*	4.447**	1.213	8.520**	1.906*	1.509	1.075**	4.490*

\* and \*\* Significant at 5% and 1% by the F test.

**Table 2.** Means for N content of the cob ( $N_{Cob}$ ), grain ( $N_G$ ) and total plant ( $N_T$ ) and shoot mass – lower ( $W_{LSH}$ ), mid ( $W_{MSH}$ ) and upper ( $W_{USH}$ ) –, cob weight ( $W_{Cob}$ ), plant dry weight (DW), total biomass (BM) and grain weight ( $W_G$ ), for the varieties Sol da Manhã NF and Catetão at two levels of N (10 and 100 kg/ha). Seropédica, RJ, Brazil, 1994.

Variety / N level	$N_{Cob}$	$N_G$	$N_T$	$W_{LSH}$	$W_{MSH}$	$W_{USH}$	$W_{Cob}$	DW	BM	$W_G$
				(kg/ha)						
Sol da Manhã NF/ 10 kg/ha	4.53	35.15	50.03	575	839	515	812	2.867	5.193	2.326
Sol da Manhã NF/ 100 kg/ha	8.08	94.13	116.74	1.276	1.182	591	1.628	4.677	10.585	5.907
Catetão / 10 kg/ha	2.58	17.20	34.35	1.537	781	489	399	3.408	4.541	1.133
Catetão / 100 kg/ha	4.87	35.93	63.76	2.231	1.590	671	845	5.370	7.563	2.224
Mean	5.01	45.60	66.22	1.405	1.098	567	921	4.080	6.970	2.897
LSD (5%)	1.73	14.77	18.64	656	488	189	261	1.129	1.576	836
Mean for Sol da Manhã NF	6.31**	64.64**	83.38**	925	1.011	553	1.220**	3.772	7.889**	4.116**
Mean for Catetão	3.72	26.56	49.06	1.884**	1.186	580	622	4.389	6.052	1.678
Mean for 10 kg/ha of N	3.55	26.17	42.19	1.056	810	502	605	3.138	4.867	1.729
Mean for 100 kg/ha of N	6.48**	65.03**	90.25**	1.754**	1.386**	631	1.237**	5.023**	9.074**	4.065**

\*\* Significant at 1% by the F test.

tion of the varieties Sol da Manhã NF and Catetão as to the N use efficiency (Tables 1 and 2).

The relative dry weights for the lower, mid and upper regions of the plant revealed about three times more dry matter in the lower region of the variety Catetão, than Sol da Manhã NF at the lower level of N and twofold the level of N (Table 2). The variety Sol da Manhã NF, at the lower N level, accumulated more dry matter at the mid region, whereas the lower and upper regions were similar for both genotypes. At the higher level of N the lower and mid regions were greater. Sol da Manhã NF presented an accentuated redistribution of dry matter at different N levels, indicating the existence of a biochemical and physiological mechanism controlling the source-sink ratio that would favor its adaptation to N stress situations. Also, Sol da Manhã NF presented much higher cob weight than Catetão at both levels of N.

Catetão had lower values for grain weight, than Sol da Manhã NF, at both N levels (Table 2). Moll et al. (1994), using the reciprocal selection method, obtained similar results, using two varieties of temperate germplasm contrasting in N efficiency. Esteves (1985) observed that maize inbreds and hybrids efficient in the use of N were those that presented higher values for N content and dry weight, and suggested these parameters as suitable for efficiency evaluation.

The data obtained in this study show that the Sol da Manhã NF variety presented lower values for total plant dry matter and higher values for grain production, compared to Catetão (Table 2). This suggests that total dry matter may not be a reliable criterion for N use efficiency in this case. It should be

pointed out that both Sol da Manhã NF and Catetão were efficient in absorbing N, but had different distribution pattern for N in the plant and had different efficiency for N translocation to the grain.

Biomass was higher for the Sol da Manhã NF than for Catetão, at both levels of N (Table 2). The components that most contributed to the biomass value of Sol da Manhã NF were the grain weight and to a lesser extent weight of the mid region and the cob, whereas for Catetão the lower shoot region contributed most to biomass, followed by the grain weight (Table 3). With regard to total plant N the main contributing components for Sol da Manhã NF were the grain and, to a lesser extent, the mid region of the shoot (Table 4). It is noteworthy that the accumulation of N in the lower, mid and upper regions together with the husks was far superior for Catetão compared to Sol da Manhã NF. The inverse occurred for N accumulation in the grain, this confirms the greater capacity of Sol da Manhã variety for N translocation to grain.

The N use efficiency ( $W_G/N_s$ ) and absorption ( $N_T/N_s$ ) were effective in discriminating the varieties only at the low N level, whereas the components of utilization ( $W_G/N_T$ ) and translocation ( $N_G/N_T$ ) were effective at both levels of N (Table 5). Therefore, the components  $W_G/N_s$ ;  $N_T/N_s$ ;  $W_G/N_T$  and  $N_G/N_T$ , at low level N, and  $W_G/N_T$  and  $N_G/N_T$ , at high level N, may be used as parameters for selection in genetic improvement programs aimed at efficient N use. These components, can help as auxiliary parameters in choosing superior genotypes in plant breeding programs and are easy to measure.

**Table 3.** Distribution of dry matter in percentage lower shoot weight ( $W_{LSh}$ ); mid shoot weight ( $W_{MSh}$ ); upper shoot weight ( $W_{USh}$ ); cob ( $W_{Cob}$ ) and grain ( $W_G$ ) in relation to total biomass (BM), for the varieties Sol da Manhã NF and Catetão at two levels of N (10 e 100 kg /ha). Calculated from the data of Table 5.

Variety / N level	$W_{LSh}$	$W_{MSh}$	$W_{USh}$	$W_{Cob}$	$W_G$
Sol da Manhã NF/ 10 kg/ha	11.07	16.15	9.91	15.63	44.79
Sol da Manhã NF/ 100 kg/ha	12.05	11.16	5.58	15.38	55.80
Catetão / 10 kg/ha	33.84	17.19	10.76	8.78	24.95
Catetão / 100 kg/ha	29.49	21.02	8.87	11.17	29.40
Mean	21.61	16.38	8.78	12.74	38.73



**Table 4.** Distribution of N as percent accumulation in the lower shoot ( $N_{LSh}$ ), mid shoot ( $N_{MSh}$ ), upper shoot ( $N_{USh}$ ), cob ( $N_{Cob}$ ), husk ( $N_H$ ), tassel ( $N_{Tas}$ ) and grain ( $N_G$ ) in relation to the total plant N ( $N_T$ ), for the varieties Sol da Manhã NF and Catetão at two levels of N (10 e 100 kg/ha). Calculated from the data presented in Tables 2 and 5<sup>(1)</sup>.

Variety / N level	$N_{LSh}$	$N_{MSh}$	$N_{USh}$	$N_{Cob}$	$N_H$	$N_{Tas}$	$N_G$
Sol da Manhã NF/ 10 kg/ha	4.89	9.55	6.22	9.07	1.43	1.46	70.25
Sol da Manhã NF/ 100 kg/ha	3.53	5.56	3.14	6.96	0.74	0.82	81.12
Catetão / 10 kg/ha	15.00	18.77	8.63	7.51	3.87	1.84	50.08
Catetão / 100 kg/ha	11.00	16.58	7.89	7.64	2.44	1.86	56.34
Mean	8.60	12.61	6.47	7.79	2.12	1.49	66.04

<sup>(1)</sup>  $N_{LSh}$ : N lower leaf + lower stem;  $N_{MSh}$ : N mid leaf + mid stem + husk;  $N_{USh}$ : N upper leaf + upper stem + tassel.

**Table 5.** Estimate of the components of absorption efficiency ( $N_T/N_s$ ), the use ( $W_G/N_s$ ), utilization ( $W_G/N_T$  and  $W_G/N_G$ ) and translocation ( $N_G/N_T$ ) of nitrogen for the varieties Sol da Manhã NF and Catetão at two levels of nitrogen (10 e 100 kg/ha). Seropédica, RJ, Brazil, 1994<sup>(1)</sup>.

Variety / N level	$N_T/N_s$	$W_G/N_s$	$W_G/N_T$	$W_G/N_G$	$N_G/N_T$
Sol da Manhã NF/ 10 kg/ha	5.00	44.74	232.60	64.91	0.68
Sol da Manhã NF/ 100 kg/ha	1.16	51.16	59.07	63.67	0.80
Catetão / 10 kg/ha	3.43	32.58	113.30	66.28	0.49
Catetão / 100 kg/ha	0.63	35.12	22.24	61.56	0.56
Mean	2.51	40.88	106.81	64.10	0.63
LSD (5%)	1.18	10.15	87.10	10.23	0.10
Mean for Sol da Manhã NF	3.08*	47.95**	145.84**	64.29	0.74**
Mean for Catetão	2.03	33.82	67.77	63.92	0.52
Mean for 10 kg/ha of N	4.21**	38.63	172.96**	65.59	0.58
Mean for 100 kg/ha of N	0.89	43.14	40.65	62.62	0.68*

<sup>(1)</sup>  $N_T$ : total plant N;  $N_s$ : N supplied (10 e 100 kg/ha);  $W_G$ : grain weight;  $N_G$ : total grain N. \* and \*\* Significant at 5% and 1% by the F test.

Translocation stands out as one of the main process underlying N use efficiency, which may mean that metabolic processes involved in synthesis can be important. The incorporation of N into protein, initiating with enzymes of  $NH_4^+$  assimilation (glutamine synthetase and glutamate synthase), might therefore play a key role in N use efficiency.

Nitrate reductase activity did not differentiate the efficient and inefficient varieties (Table 6). In the greenhouse, experiment activity was affected by N level, but within each level the two varieties behaved similarly.

Eichelberger et al. (1989a, 1989b) reached at similar conclusions with the stiff-stalk population, where

again selection for higher enzyme activity did not lead to greater yields. Purcino et al. (1994) evaluated nitrate reductase activity in ancient and modern maize genotypes, grown under two levels of N, and found that the modern varieties presented better yields but that nitrate reductase activities measured at three different stages did not correlate with grain yield.

Another key enzyme of N assimilation studied here is glutamine synthetase (GS). No significant differences were found between the two varieties for activity of this enzyme at both levels of N (Table 6.) In the greenhouse experiment, the roots showed higher activities at the higher N level while in the leaves the tendency was to be higher in the low N level.

**Table 6.** Mean activities of glutamine synthetase (GS) and nitrate reductase (NR) from the field and greenhouse experiments for the varieties Sol da Manhã NF and Catetão at two levels of N (10 and 100 kg/ha for the field experiment). Data expressed as  $\mu\text{mol/h/g}$  of fresh weight. Seropédica, RJ, Brazil, 1994, and Campinas, SP, Brazil, 1995.

Variety / N level	Field		Greenhouse		
	GS (leaf)	NR (leaf)	NR (leaf)	GS (leaf)	GS (root)
Sol da Manhã NF/ 10 kg/ha	457	0.93	0.22	233	241
Sol da Manhã NF/ 100 kg/ha	452	1.68	0.74	217	303
Catetão / 10 kg/ha	478	1.16	0.26	260	251
Catetão / 100 kg/ha	493	1.34	1.14	219	336
Mean	470	1.28	0.59	232	283
LSD (5%)	110	0.89	0.38	48.50	41.77
Mean for Sol da Manhã NF	454	1.30	0.48	225	272
Mean for Catetão	485	1.25	0.70	239	293
Mean for 10 kg/ha of N	467	1.04	0.24	246	246
Mean for 100 kg/ha of N	473	1.51	0.94**	218	319**

\*\* Significant at 1% by the F test.

## Conclusions

1. The differential translocation of N and photoassimilates to the grain are important to distinguish between varieties efficient and non-efficient in the N use.

2. The Sol da Manhã NF and Catetão varieties are efficient and non-efficient in the N use, respectively.

3. The source/sink ratio is different in the Sol da Manhã NF and Catetão varieties. Sink is the most important parameter for identifying the variety of N efficient use.

4. Grain and total N content, cob weight and the ratio of ear dry weight to total dry weight can be used as auxiliary selection parameters for maize breeding programs.

5. The varieties Sol da Manhã NF and Catetão, in spite of presenting common germplasm, both with predominance of the Cateto race, have different performance in the N use, indicating high variability into this race to this parameter.

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